

Triple-Negative Breast Cancer: Risk Factors to Potential Targets

Bryan P. Schneider,¹ Eric P. Winer,² William D. Foulkes,³ Judy Garber,² Charles M. Perou,⁴ Andrea Richardson,² George W. Sledge,¹ and Lisa A. Carey⁴

Abstract Triple-negative breast cancer has recently been recognized as an important subgroup of breast cancer with a distinct outcome and therapeutic approach when compared with other subgroups of breast cancer. Triple-negative breast cancer comprises primarily, but not exclusively, a molecularly distinct subtype of breast cancer, the basal-like subtype. We do not yet have an assay to identify basal-like breast cancer in clinical samples, so triple-negative breast cancer has become a commonly used proxy for this subtype. The molecular biology and pathophysiology of triple-negative breast cancer are not completely understood, but understanding is improving rapidly with the advent of sophisticated molecular biology platforms. Moreover, the established risk factors of breast cancer as a whole may not apply to this unique subgroup of patients. Finally, because triple-negative breast cancer is defined by the absence of a target, there are currently limitations to using a tailored therapeutic approach, leaving conventional cytotoxic therapies as the mainstay. Active preclinical and clinical research programs focus on defining the clinical behavior, delineating the risk factors, and more completely understanding the molecular biology of triple-negative breast cancer to improve prevention, optimize conventional agents, and unveil novel therapeutic targets. This CCR focus article will review the current state of the art on triple-negative breast cancer.

Triple-negative breast cancer [estrogen receptor negative, progesterone receptor negative, human epidermal growth factor receptor 2 (HER2) negative] remains a major challenge to physicians and patients, and a source of great interest to laboratory investigators. Although triple-negative breast cancer accounts for a relatively small minority of breast cancer cases, it is responsible for a disproportionate number of breast cancer deaths. Moreover, there have been fewer advances in the treatment of triple-negative breast cancer than have been seen with other subtypes. For these reasons, new research initiatives for triple-negative breast cancer are critical. The investigation of triple-negative breast cancer is one facet of an emerging effort that regards breast cancer as a collection of separate diseases rather than a single heterogeneous entity, an important step toward the individualization of therapy (1). Here we attempt to (a) define triple-negative breast cancer and compare and contrast it with basal-like disease (a term frequently used interchangeably with triple-negative disease); (b) outline established and proposed risk factors; (c) review the molecular, pathologic, and clinical features of triple-negative disease; (d)

provide an overview of ongoing therapeutic trials; and (e) suggest possible avenues for future research.

Triple-Negative or Basal-like Breast Cancer: Which Is It?

Triple-negative breast cancer has become a commonly used descriptor for malignancies that are estrogen receptor, progesterone receptor, and HER2 negative. The recent focus on this subgroup of tumors has arisen for two major reasons. First, unlike tumors that are estrogen receptor and/or HER2 positive, triple-negative tumors lack an established therapeutic target. As a result, conventional chemotherapy is the only effective systemic treatment for these patients and there is an urgent need for new treatment approaches. Second, recent developments in gene expression arrays have categorized breast cancer into distinct subgroups. One of these subgroups as defined by genetic clustering is the basal-like group of tumors (refs. 2, 3; Fig. 1). Among the features of this basal-like subgroup defined by gene expression pattern is low expression of hormone receptor- and HER2-related genes, so most of these are triple-negative breast cancers. Because gene array profiling is not clinically available, and immunohistochemical surrogate profiles for the basal-like profile have not been standardized or validated (4), clinicians do not have either direct or indirect access to the molecular subtype. For this reason, breast cancers in the clinical setting are more typically categorized by routine immunohistochemistry as triple-negative breast cancer as a proxy for the basal-like subtype. It is crucial to note, however, that although most basal-like cancers are triple-negative breast cancer, there is moderate discordance between triple-negative breast cancer and basal-like breast cancer

Authors' Affiliations: ¹Indiana University Melvin and Bren Simon Cancer Center, Indianapolis, Indiana; ²The Dana-Farber Cancer Institute, Boston, Massachusetts; ³Program in Cancer Genetics, McGill University, Montreal, Canada; and ⁴University of North Carolina, Chapel Hill, North Carolina
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Requests for reprints: Bryan P. Schneider, Indiana Cancer Pavilion, Room 473, 535 Barnhill Drive, Indianapolis, IN 46202. Phone: 317-274-6473; Fax: 317-274-3646; E-mail: bpschnei@iupui.edu.

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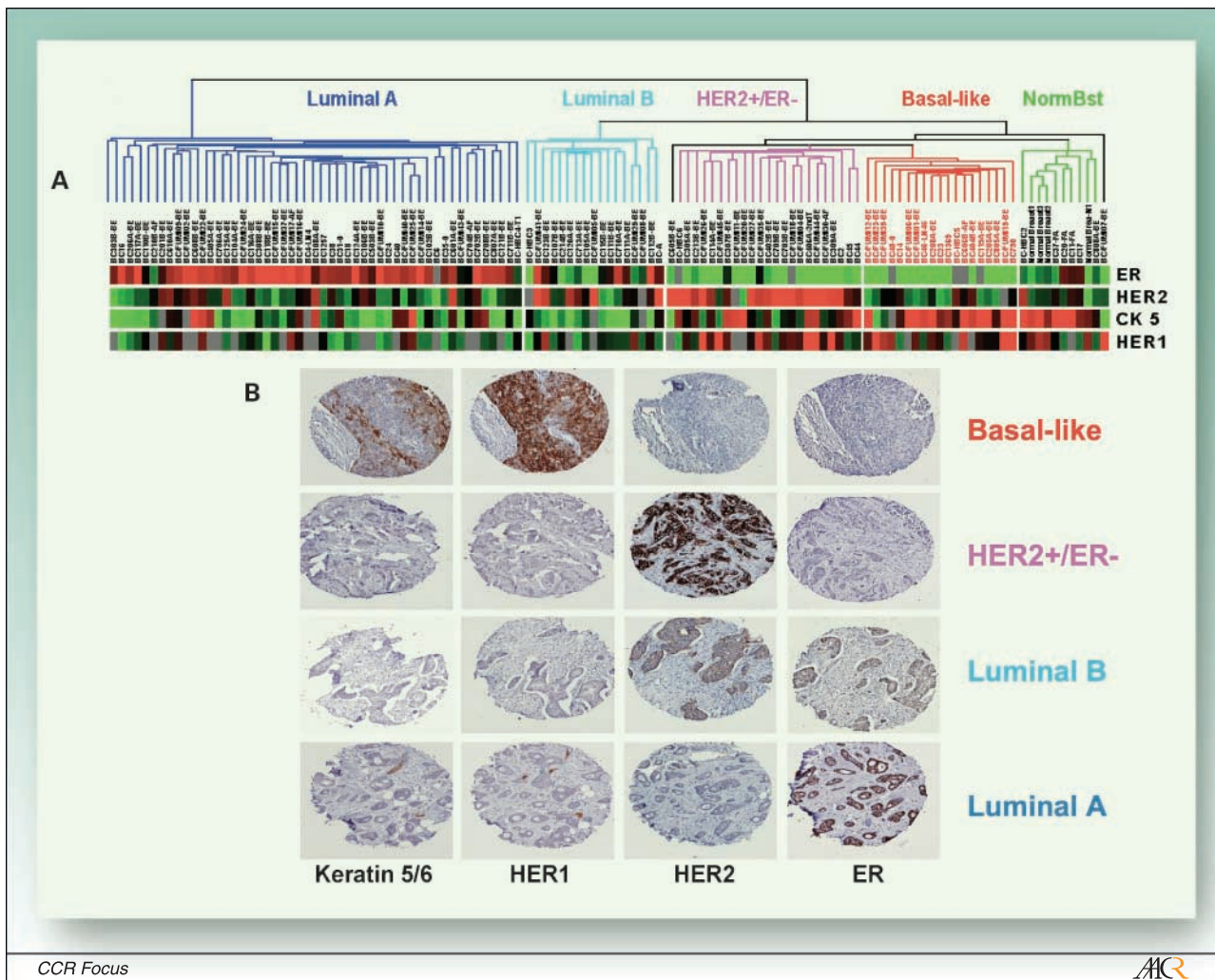


Fig. 1. Immunohistochemical surrogate assay for intrinsic subtypes of breast cancer. *A*, hierarchical cluster dendrogram taken from Carey et al. (2) and color-coded according to subtype, showing the gene expression data for the four genes/proteins used for immunohistochemical classification. Adapted with permission from the *Journal of the American Medical Association*, 295(21):2492–2502. Copyright © 2006 American Medical Association. All rights reserved. *B*, tissue microarray results for the representative immunohistochemical profiles used in Nielsen et al. (4) to identify the intrinsic subtypes.

(5, 6). In addition to variability in expression of known basal markers, there is also heterogeneity within triple-negative breast cancer for other potentially relevant features including *p53* mutation, *BRCA1* mutation or expression (7), expression of α -B-crystallin (8, 9), and degree of expression of immune response genes (refs. 5, 10; Fig. 2). Because of this discordance and potential for misclassification, in this review we will refer to basal-like breast cancer when gene expression array or more sophisticated immunophenotypes were used for identification and triple-negative breast cancer when the analysis was limited to clinical assays.

Basal-like Breast Cancer

Gene expression arrays have reproducibly identified breast cancer molecular subtypes with clinical and prognostic implications. These studies confirmed the importance of

hormone receptors and HER2 as central to the biological variance among breast cancers, and have provided additional data about the biology of these subtypes. One of the subtypes is the basal-like. The term “basal-like” breast cancer comes from the resemblance of its expression pattern to that of the myoepithelial cell of the breast, but this definition does not necessarily imply that basal-like tumors arise from these cells. From a historical perspective, this subgroup was identified long ago by studies examining phenotype and cytokeratin profiles (11, 12). Using gene expression profiling or immunohistochemical surrogates, this subgroup of tumors are notable not only for low expression of hormone receptor- and HER2-related genes but also for high expression of proliferative genes, including *Ki-67*, and for expression of a characteristic “basal-like” gene cluster (2, 3, 13), which includes high expression of basal cytokeratins (CK) such as CK5 and 17, as well as caveolin-1, the

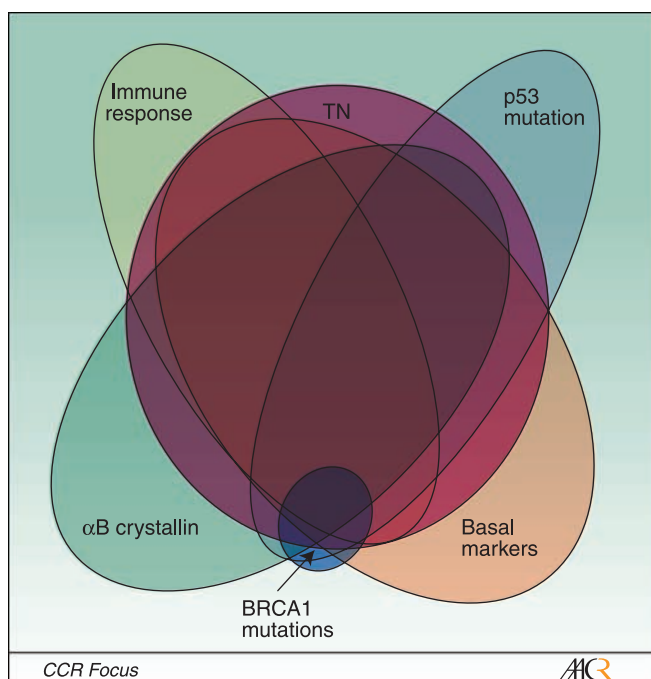


Fig. 2. Image showing the relationship and overlap between breast cancer subgroups that share characteristics. Triple-negative (TN) breast cancers are in pink. It can be seen that breast cancers expressing basal markers, such as CK5 and EGFR (dark tan), overlap considerably with TN breast cancers, but the overlap is not complete and not all TN tumors are positive for these markers. These “nonbasal” TN tumors may be the result of technical failures in estrogen receptor and HER2 testing (falsely “triple-negative”) or may be ultimately shown to have basal features as additional markers of the basal phenotype are discovered and accepted. A subset of TN tumors has an expression profile indicating a strong immune response, presumably to breast cancer cell surface antigens (light tan), a feature that may have prognostic implications within the TN cancers. There are additional molecular features that characterize some but not all TN tumors including but not limited to *p53* mutation (light blue), *BRCA1* germ-line mutation (dark blue), and α B crystallin expression (light green). Which of these various subsets of TN cancer will have clinical differences or treatment implications is yet to be determined.

epidermal growth factor receptor (EGFR), α B-crystallin, P-cadherin, and c-KIT (Fig. 3). Other notable characteristics of this subtype include frequent *p53* mutations (2, 13), gene copy number aberrations and evidence of genomic instability (14–17), and Rb pathway inactivation (Fig. 4; refs. 18, 19).

There is an intriguing association of basal-like breast cancer with germ-line *BRCA1* mutations (13, 20), one of the most important forms of hereditary breast cancer. At least three quarters of *BRCA1*-related breast cancers are basal-like by microarray (13) or by immunohistochemistry (21). Genomic instability seems characteristic of both *BRCA1*-related breast cancer and basal-like breast cancer, which may reflect aberrant DNA repair pathways that are common to both subtypes of cancer (22). Indeed, they are, in general, remarkably similar whether the perspective is DNA, RNA, or protein. For this reason, several investigators are exploring the role of the *BRCA1* pathway in sporadic basal-like cancers. What has become clear is that the relationship is not simple (23). For example, a classic two-hit theory of carcinogenesis does not apply to *BRCA1* and basal-like breast cancer.

Although *BRCA1* is inactivated by one mechanism or another in at least some basal-like breast cancer, somatic *BRCA1* mutations are very rare in breast cancer (24, 25). Methylation of the promoter of *BRCA1* has been found (mainly in medullary and metaplastic breast cancers; refs. 7, 26, 27), but there are conflicting data (7, 28), especially if the studies are limited to basal-like breast cancers that do not fall into these two categories. Moreover, decreased expression of *BRCA1* in basal-like breast cancer was seen in one study (7) but not in another (29). In the latter study, one common factor seen in both basal-like breast cancer and *BRCA1*-related breast cancer was a defect in the maintenance of normal chromosome X-inactivation. Thus, a complex picture emerges: it seems likely that there is considerable heterogeneity within basal-like breast cancer, as has been suggested by microarray

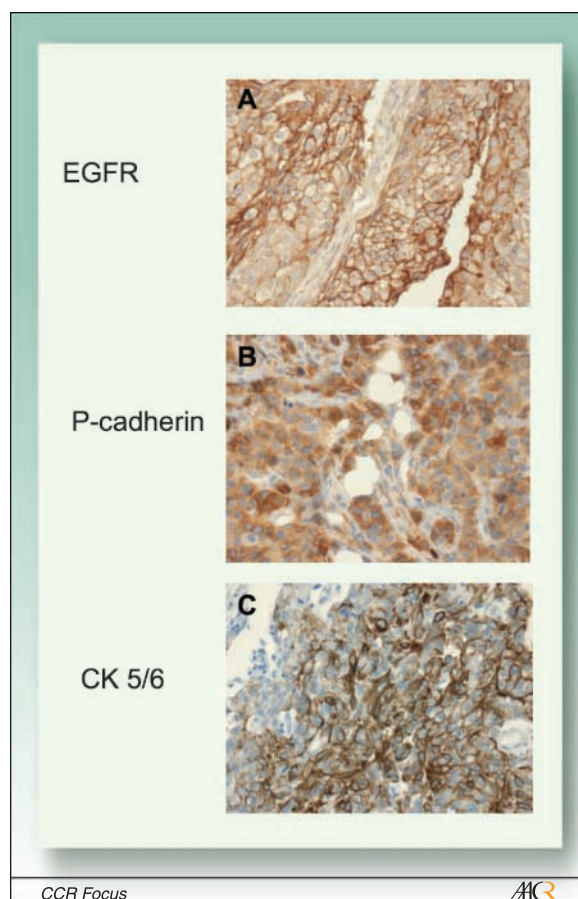


Fig. 3. Three “basal” breast cancer markers. Examples of three basal markers that are characteristic of many triple-negative breast cancers. Note the lymphocytic infiltration, indicative of the presence of tumor-infiltrating lymphocytes in C (Images courtesy of Dr. Lars Akslen and Dr. Jarle Arnes, The Gade Institute, Section for Pathology, Haukeland University Hospital, University of Bergen, Bergen, Norway). Of note, the absence of these three, or any other specified markers, does not by definition make the tumor “nonbasal.” A, strong membrane expression of EGFR in a poorly differentiated ductal carcinoma (Nottingham grade 3; $\times 400$). B, strong membrane and cytoplasmic expression of P-cadherin in an invasive ductal carcinoma (Nottingham grade 3; $\times 400$). C, strong expression of cytokeratin 5/6 in a poorly differentiated breast carcinoma with lymphocytic infiltration (Nottingham grade 3; $\times 400$).

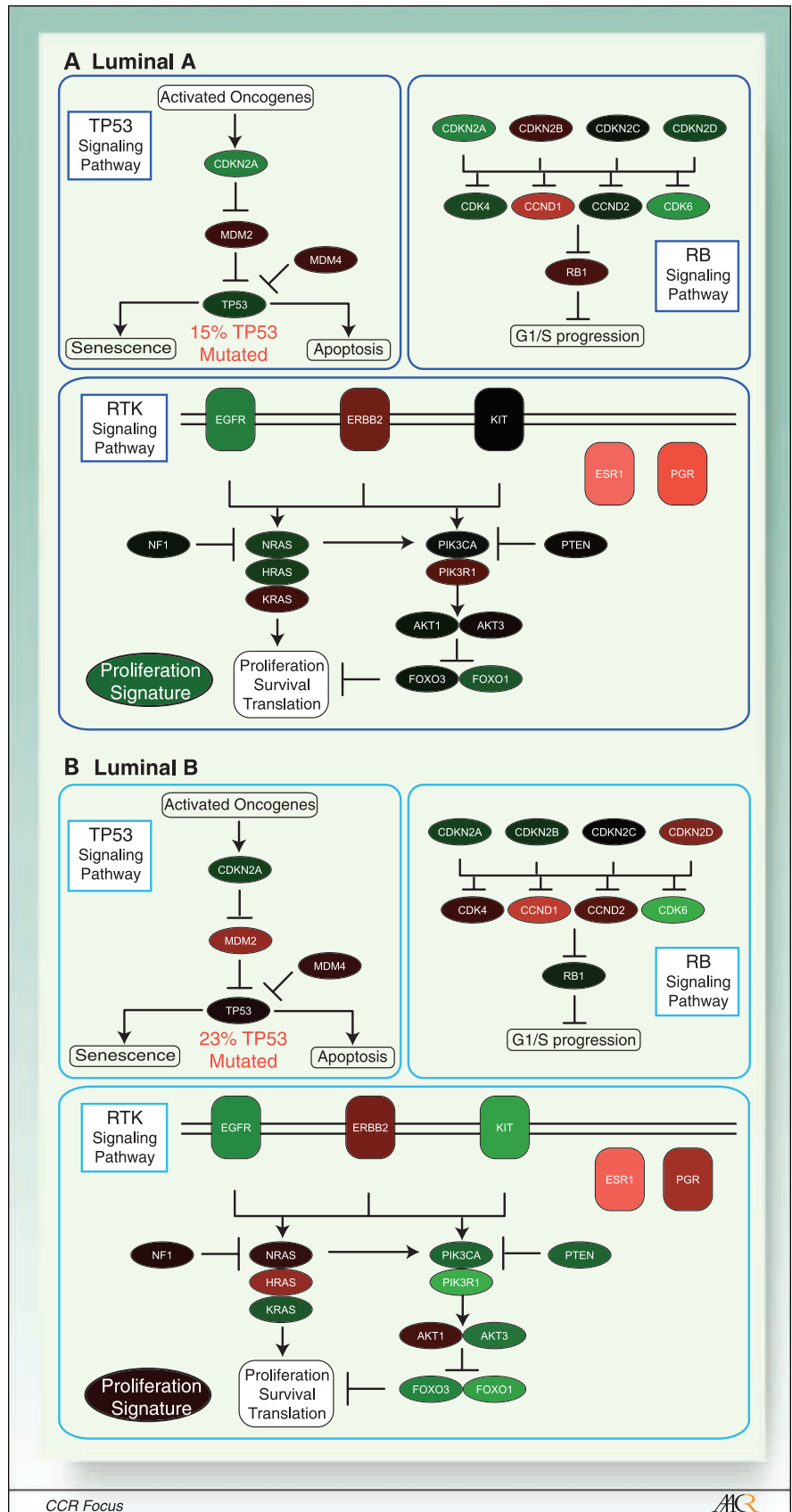


Fig. 4. Pathway analysis of the intrinsic subtypes. TP53, retinoblastoma, and receptor tyrosine kinase signaling pathways are shown for many of the major genes within each pathway. Each gene is color-coded according to the average expression of that gene with each subtype (61). An average value for the "proliferation signature" for each subtype is also shown within the receptor tyrosine kinase (*RTK*) pathway box. TP53 mutation status is also shown for each subtype (2). These analyses highlight the triple-negative phenotype, the high expression of EGFR and c-KIT in basal-like tumors, and show their high TP53 mutation rates. Pathway analyses are shown for (A) luminal A and (B) luminal B.

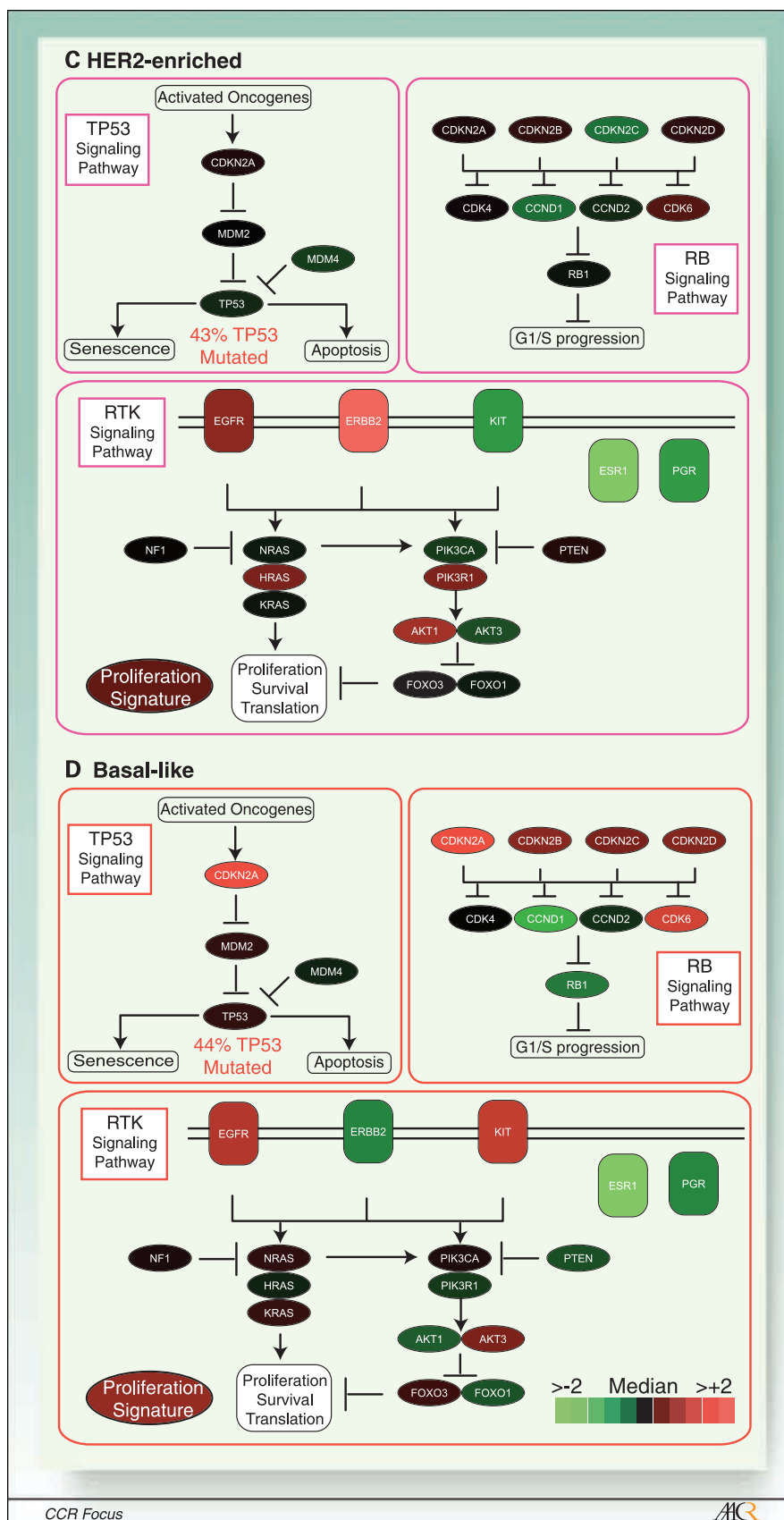


Fig. 4 Continued. Pathway analyses are shown for (C) HER2-enriched and (D) basal-like tumors.

studies (ref. 30; Fig. 2). *BRCA1*-related breast cancers are also not homogeneous: mice conditionally mutant for *Brca1* developed at least three different molecular and histologic subtypes of breast cancer (31). This is mirrored in humans as not all breast cancers arising in *BRCA1* mutation carriers are triple-negative breast cancers or basal-like breast cancers. Interestingly, the proportion of estrogen receptor-negative tumors in women with *BRCA1* mutations decreases with age. Approximately 20% of those younger than 45 years of age at diagnosis have estrogen receptor-positive disease compared with ~40% for those 55 to 65 years of age (32).

The clinical outcomes for women with sporadic, basal-like breast cancer compared with those with *BRCA1*-related cancers are broadly similar, and notable for early (within 5 years) relapse. In addition to the timing of relapse, the pattern of metastatic spread is also similar for *BRCA1*-related (33) and basal-like breast cancer (34). The characteristics of hereditary *BRCA1*-associated breast cancer found in sporadic cancers has been termed "BRCA-ness," with potential clinical implications as described further below (22).

Risk Factors for Basal-like Breast Cancer

BRCA1 mutation confers an exceptionally high risk of developing basal-like breast cancer, but there may be many other lower-penetrance genes that raise this risk either alone or in concert with other genes or with environmental exposures. Identifying these genes will require large-scale genetic association studies such as genome-wide association studies, which are in development. Although not yet specifically designed to study by subtype, a recent genome-wide association study uncovered several powerful susceptibility loci for unselected breast cancers (35). These included five novel susceptibility loci that had never previously been reported in association studies. These included single-nucleotide polymorphisms in *FGR2*, chromosome 8q, *CASP8*, *TNRC9*, *MAP3K1*, and *LSP1* (35). Very weak associations confined to estrogen receptor-positive breast cancers were also identified with single-nucleotide polymorphisms from 16q12 and 2q35 (36). To date, the majority of these associations seem to be more significantly correlated with estrogen receptor-positive tumors (37, 38). The use of these powerful genetic platforms, applied to more selected populations, will help to understand the biology of basal-like breast cancer and hopefully provide insight into preventive approaches.

Several studies suggest that breast cancer subtypes vary by race and age; premenopausal women and African American women are far more likely to develop basal-like, and far less likely to develop luminal A breast cancers than their postmenopausal and white counterparts (2, 39–42). For example, in African American women, there is a near doubling of the percentage of basal-like breast cancer (20–27%) when compared with Caucasian women (10–14%; refs. 2, 39, 41, 42). Although none of the older epidemiologic studies were designed to identify risk factors by molecular subtype, recent post hoc reanalyses also raise interesting questions about traditional risk factors and whether some risk factors are

stronger for one subtype versus another or even have opposite effects in different subtypes. For example, in contradistinction to luminal breast cancer, higher parity and young age at first birth may be risk factors for basal-like breast cancer, whereas lack of breast feeding and early age of menarche may be stronger risk factors than for luminal breast cancers (41, 43, 44). Given our understanding of breast cancer as a heterogeneous disease, further research will need to focus on examination of individual risk factors within subtypes.

Clinical Characteristic and Outcomes of Basal-like Breast Cancer

There are several consistent clinical characteristics of this subtype. The basal-like group accounts for ~15% of all invasive breast cancers. Pathologically, most basal-like breast cancers possess high histologic and nuclear grade, poor tubule formation, high mitotic and proliferative indices, and can have a pushing border. Most are infiltrating ductal, although some unusual histologies such as metaplastic breast cancer share features of basal-like breast cancer (45). Despite the relatively poor prognosis noted in historical data sets, this subtype presents at generally similar stage to other subtypes (2), but the highly proliferative nature of this tumor may explain the association with interval (becoming clinically detectable between mammograms) cancer (46).

Multiple data sets have consistently identified a poorer clinical outcome for women with basal-like breast cancer (47), although modern chemotherapy may alter this history. Among triple-negative tumors, the risk of recurrence is higher in the first 3 to 5 years after diagnosis than in women with estrogen receptor-positive tumors, with relatively few systemic recurrences after the first 5 years, suggesting that a substantial number of women are cured if they remain recurrence-free for the first several years after a diagnosis (48, 49).

Because triple-negative breast cancers constitute a group of tumors without targeted therapeutics, clinicians are left to rely on relatively nonspecific cytotoxic agents. Nevertheless, cytotoxic therapy can be quite effective in patients with triple-negative breast cancer. A retrospective evaluation of CALGB 9344 found that patients with either triple-negative or HER2-positive breast cancer derived the greatest benefit from the addition of paclitaxel (T) to doxorubicin and cyclophosphamide (AC; ref. 50). Similarly, dose-dense therapy seems to have the greatest incremental benefit in women with estrogen receptor-negative tumors (51). Preoperative studies also suggest sensitivity to modern anthracycline/taxane-based regimens as measured by a high pathologic complete response in basal-like breast cancer or triple-negative breast cancer (52). Although this seemed paradoxical given the poor prognosis associated with basal-like breast cancer/triple-negative breast cancer, studies suggest that pathologic complete response carries a good outcome regardless of subtype. However, patients with basal-like breast cancer or triple-negative breast cancer who do not achieve a pathologic complete response were more likely to relapse early than luminal or hormone receptor-positive breast cancers (52). In summary, women with primary basal-like tumors seem to have a high likelihood of response to

chemotherapy, but if the disease is not chemosensitive, they have a worse outlook given the absence of known targetable molecules and the reliance on chemotherapy alone. Improved identification of those for whom modern adjuvant therapy is inadequate may rely on approaches such as gene expression analysis to identify a panel predictive of pathologic complete response to T→FAC (5-fluorouracil, doxorubicin, cyclophosphamide; ref. 52) or a panel that identifies regimen-specific signatures such as those developed for FEC (5-fluorouracil, epirubicin, cyclophosphamide) versus docetaxel followed by the combination of docetaxel with epirubicin (53).

Given the importance of chemotherapy in this disease, investigators have focused on optimizing drug selection of existing chemotherapeutic agents. From a biological standpoint, DNA-damaging agents (such as platinating agents) are of a high priority based on the *BRCA1* pathway and DNA repair dysfunction in this subtype as described above, which may confer enhanced sensitivity to DNA-damaging agents. There are very few prospective studies that have specifically focused on patients with triple-negative disease. Only small studies exist, but are supportive of this approach, including a neoadjuvant trial of single agent cisplatin in 28 women with triple-negative disease showing 22% pathologic complete response to the single agent (54), and another neoadjuvant trial of women with *BRCA1* mutations and triple-negative breast cancer, in which 9 of 10 had pathologic complete response to single agent cisplatin (55). In two platinum-based regimens in the pretreated metastatic setting, response rates of 17% to carboplatin plus cetuximab (56) and 30% to carboplatin plus irinotecan (57) were seen. These results further support research into the utility of platinating agents in triple-negative breast cancer, but clearly there is a need for additional studies before cisplatin or any similar agent can be considered standard for frontline therapy. The additional benefit of carboplatin added to paclitaxel in triple-negative disease will be directly studied in the neoadjuvant trial CALGB 40603. There is little question, however, that better therapies are needed. Given the lack of targeted therapy for triple-negative breast cancer, strategies that maximize the benefits associated with standard cytotoxic therapy could lead to further reductions in breast cancer recurrence and mortality in women with triple-negative disease.

The promise of a targeted approach in this subtype of breast cancer is real, particularly with antiangiogenic strategies. ECOG 2100, a phase III North American Breast Cancer Intergroup trial, randomized over 700 women who had never received chemotherapy in the metastatic setting to paclitaxel with or without bevacizumab (58). There was a significant improvement in the primary end point of progression-free survival with the addition of bevacizumab overall, including the subgroup of patients with largely triple-negative disease. In this context, it is notable that *BRCA1*-related breast cancers are associated with the presence of glomeruloid microvascular proliferation, a marker of increased neoangiogenesis in cancer (59). Another recently reported study of the multitargeted vascular endothelial growth factor receptor inhibitor sunitinib suggested a response rate of ~15% in the pretreated triple-negative subset of patients (60). This approach will be tested directly in the neoadjuvant setting in CALGB 40603, which

includes a second randomization to receive or not receive preoperative bevacizumab in addition to the assigned chemotherapy.

Based on EGFR expression in gene profiling studies and EGFR dependence for growth and proliferation on basal-like breast cancer cell lines (61), several groups have examined EGFR targeting in triple-negative breast cancer. Two studies completed to date shed light on this approach. TBCRC 001 was a randomized phase II trial evaluating the role of EGFR inhibition for triple-negative metastatic breast cancer. In this study, eligible women received the anti-EGFR monoclonal antibody cetuximab combined with carboplatin, or cetuximab alone with a planned crossover to carboplatin at progression. Not surprisingly, cetuximab alone showed a low response rate and was closed early by design, but response to the combination of cetuximab plus carboplatin was 17% with clinical benefit seen in 29% of a pretreated population (56). A similar study examining irinotecan plus carboplatin with or without cetuximab suggested a modestly higher response rate (from 30% to 49%) with the combination in triple-negative breast cancers on subset analysis (57).

The Current Plan of Attack

The optimal therapeutic approach for triple-negative breast cancer will likely include a mixture of targeting the host via an intimate understanding of pharmacogenetics (62) and targeting the molecular biology of the tumor. Based on attempts to pair the molecular biology of triple-negative breast cancer with drug mechanism, multiple compounds are in testing for triple-negative breast cancer. These include taxanes, platinating agents, antiangiogenic agents, EGFR inhibitors, poly(ADP-ribose) polymerase inhibitors, and Src-Abl inhibitors, among others. A number of trials have begun that are targeting this subgroup. As mentioned earlier, CALGB 40603 is a neoadjuvant trial specifically designed for patients with triple-negative disease and will address two very timely questions. It will attempt to discern the true value of adding either or both a platinating agent and an antiangiogenic therapy to traditional chemotherapy in a 2 × 2 randomization schema. Another focus continues to be the role of EGFR in basal-like breast cancer. In TBCRC 001, in which women with triple-negative breast cancer received carboplatin plus cetuximab as described above, gene expression studies were done on serial biopsies of the target lesions in 16 women. These studies suggest that EGFR targeting alone may be effective in some, but is insufficient in the majority of triple-negative breast cancers, in whom other agents may be needed for pathway inhibition. The vascular endothelial growth factor receptor-2 small-molecule tyrosine kinase inhibitor sunitinib is being studied in the advanced setting where patients are randomized to sunitinib versus standard of care. Other novel agents of interest include the multitargeted Src-Abl inhibitor dasatinib. A cell line-derived predictive model of sensitivity to dasatinib applied to expression profiles from human tumors overlapped significantly with the triple-negative breast cancer tumors (63), leading to a recently completed but not yet reported phase II trial in this subtype. Poly(ADP-ribose) polymerases are molecules integrally

involved in nonhomologous DNA repair, which become the primary means of double-strand DNA repair when the preferred homologous recombination mechanism is lost, as occurs when the BRCA1 pathway is defective. BRCA1 loss or inactivation thus sensitizes cells to poly(ADP-ribose) polymerase inhibitors (64). For the reasons described above, both hereditary and at least a subset of sporadic basal-like breast cancer are thought to have dysfunctional BRCA1 pathways, resulting in several phase II studies of poly(ADP-ribose) polymerase inhibitors alone and in combination with DNA-damaging agents in both BRCA1 carriers and triple-negative breast cancer. In addition to the drug selection based on an intimate understanding of the molecular biology of triple-negative breast cancer (as described above), the development of companion predictive markers will ultimately optimize the therapeutic success for this subgroup (65).

Summary

Although the risk factors for estrogen receptor-positive disease are well defined, those for triple-negative disease are less well defined. A more comprehensive understanding of gene expression and genetic variability is central to understand both the etiology and pathogenesis of this disease. A validated risk model (similar to the modified Gail model) for triple-negative disease would be clinically useful. Such a model might help facilitate the development of chemopreventive agents or lifestyle modifications. With regard to our current approach to therapy, triple-negative breast cancer should be treated with conventional therapies in the curative setting at this time. Incorporation of platinating agents and other novel therapeutics, although promising in the *in vitro* and advanced setting, should await further data from clinical trials. Retrospective correlative strategies focusing on the triple-negative breast cancer subgroup may help identify which subgroups will benefit most from standard drugs. Prospective trials are now under way, designed to study a given regimen by subgroup of disease (usually estrogen receptor-positive versus HER2-positive versus triple-negative breast cancer). Simultaneously,

novel agents are being evaluated in the advanced setting and added to more traditional regimens in the curative setting.

In the absence of agreed standards for staining and scoring of basal markers using immunohistochemistry, the coming generation of trials for patients with basal-like breast cancer/triple-negative breast cancer should continue to classify the disease on the basis of estrogen receptor/progesterone receptor and HER status. At the present, these tests are commonly used, represent relatively inexpensive biomarkers, and will allow for rapid conversion of results into clinical application. We recognize, however, that current and future gene array platforms have the power to yield a more thorough prediction of the molecular biology (which has the capacity to identify novel targets) and a greater sensitivity for distinguishing various subtypes of disease. At the current time, we believe that trials should focus on recruitment of triple-negative breast cancer but of a sufficiently large population to have statistical power to retrospectively analyze for a molecularly defined subgroup as well.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

On December 11, 2007, the Triple Negative Breast Cancer Foundation and Susan G. Komen for the Cure convened a meeting of clinicians, investigators, and advocates to review the state of current clinical and translational research on triple-negative breast cancer and make recommendations regarding opportunities for research in this breast cancer subtype. This is a report derived from that meeting. We acknowledge and sincerely thank the Triple Negative Breast Cancer Foundation and the Susan G. Komen for the Cure for their support of this symposium.

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